23. Waksman, S. A., Soil Science 8, 168 (1919).
24. Griffon, E. and Maublanc, A., Bull. Soc. Mycol. France 27, 68 (1911).
25. Tsiklinsky, R., Ann. Inst. Pasteur 13, 500 (1899).
26. Lechevalier, H., Solotorovsky, M., and McDurmont, C., J. Gen. Microbiol. 26, 11 (1961).
27. Cross, T., Maciner, A. W., and Lacey, J., J. Gen. Microbiol. 50, 351 (1968).
28. Gregory, P. H., Festenstein, G. N., Lacey, M. E., and Skinner, F. A., J. Gen. Microbiol. 26, 429 (1964).

Received May 13, 1968. P.S.E.B.M., 1968, Vol. 129.

Heterogeneity of Antisera to Human Growth Hormone, Demonstrated by the Immunofluorescence Technique* (33320)

R. NAYAK,[1] ELEANOR E. MCGARRY, AND J. C. BECK
McGill University Clinic, Royal Victoria Hospital, Montreal, Canada

Earlier studies (1) showed the fluorescein-conjugated antisera to human growth hormone (HGH) localized in acidophils of human, rat, and bovine pituitary glands, and that the fluorescence, which was inhibited when the specific antigen or the unconjugated antisera was applied before staining, was specific.

During inhibition studies, one batch of anti-HGH (titer, 1/12,000; batch HGH-5) inhibited fluorescence caused by another batch of fluorescein-conjugated anti-HGH (titer 1/25,000; Hg-1) in the human but not the rat pituitary gland. Inhibition was repeated with anti-HGH batch HGH-5 and followed by 1:2 and 1:3 dilution of fluorescein-conjugated anti-HGH Hg-1 (which still produced fluorescence); again, fluorescence was not inhibited in the rat pituitary. However, when conjugated and unconjugated anti-HGH Hg-1 from the same batch were used, staining was completely inhibited in both rat and human pituitaries. The present studies were carried out to determine the cause of this discrepancy.

Materials and Methods. The immunofluorescent-staining method used was described previously (2). Preparation of antisera to HGH from 9 rabbits and 1 guinea pig were used for inhibition experiments. Fluorescein-conjugated anti-HGH Hg-1 and Hgh-5, which were used as staining antisera, produced bright fluorescence in rat, human, and bovine pituitaries. The titers were measured by the bis-diazotized benzidine (BDB)–hemagglutination method.

Results. Two antisera did not inhibit staining in any experiment (Table I and II). In human pituitaries, all others produced inhibition of variable degree. In rat, complete inhibition occurred with only two antisera (Table I). In bovine pituitaries, complete inhibition occurred with only one antiserum. Six antisera were conjugated with fluorescein (Table III); since the titer of antisera that failed to stain was unchanged after conjugation, activity was not destroyed during this procedure. Indirect staining, using fluorescein-conjugated sheep anti–rabbit gamma globulin, showed some degree of fluorescence with all eight antisera (Table IV).

These experiments indicate that different rabbits may produce different antisera to a single antigen. Some antisera with high titers failed to inhibit staining, whereas others with lower titers produced fluorescence. When the same antiserum was used for inhibition and staining, inhibition always occurred and was complete; when different antisera were used, inhibition did not always occur. The two antisera that produced no fluorescence were against the same preparations of the antigen and it is possible that HGH batches 163,

* Supported in part by Grant HD 00511-06 from the USPHS.

[1] Medical Research Fellow of the Medical Research Council of Canada. Some of this material was included in Dr. Nayak's thesis for the Ph.D. degree in Experimental Medicine, McGill University, Montreal, 1966.

TABLE I. Inhibition Studies in Rat and Human Pituitary Glands with Various Antisera to HGH.[a]

	Inhibiting antisera (unconjugated)		HGH used for immunization	Titer of antiserum[b]	No. of booster injections	Degree of inhibition[c]	
						Rat	Human
1	Hgh-3	Rabbit	Raben[d]	1/6400	2	++	++++
2	Hgh-5	Rabbit	Raben[d]	1/6400–1/12,800	4	++++	++++
3	Hgh-10	Rabbit	Raben[d]	1/25,000	4	+	++++
4	Hg-1	Rabbit	HGH 280564	1/25,000	1	++++	++++
5	Hgh-2X	Rabbit	Nordic 163	1/6400	0	0	0
6	Hgh-3X	Rabbit	Nordic 163, 263, 363	1/6400	3	0	0
7	Hgh-4X	Rabbit	HGH 7/11/63	1/25,000	0	+	++++
8	Hgh-5X	Rabbit	HGH 28054	1/6400–1/12,000	0	0	++++
9	Hgh-6X	Rabbit	HGH 28054, 280564	1/25,000	0	+	++++
	Hgh-GP	Guinea pig	HGH 11/9/63	1/25,000	0	+	++++

[a] Fluorescein-conjugated anti-Hgh-5 was used for staining.

[b] Titers were determined by bis-diazotized benzidine (BDB) – hemagglutination method.

[c] 0 means no inhibition; ++++ complete inhibition.

[d] Kindly supplied by Dr. M. S. Raben, Deaconess Hospital, Boston, Massachusetts, and prepared by Nordic Chemicals Ltd., Montreal, Canada, by the technique of Raben (6).

263, and 363 differed from other HGH preparations in the antigenic groups exposed.

These inhibition studies indicate that different rabbits had chosen different antigenic groups against which to form antibody. Of the ten antisera to HGH, only two (Hgh-5 and Hg-1) produced complete inhibition of fluorescence in rat pituitary, whereas eight produced complete inhibition in human pituitary (Table I). In another experiment (Table II), with a different batch of anti-HGH (Hg-1), only this antiserum produced inhibition in rat and bovine pituitaries, and others inhibited only partially (in rat) or not at all (in bovine pituitary). In human, only four antisera produced complete inhibition. In both experiments with Hg-1 or Hgh-5, the two antisera (Hgh-2 and Hgh-3) inhibited staining in none of the pituitaries. The same antigen (Nordic 163) was used for immunization with both antisera and the titers were similar (1:6,400). In other rabbits injected with different batches of antigen, the antisera produced variable degrees of inhibition.

TABLE II. Inhibition Studies in Rat, Human, and Bovine Pituitary Glands with Various Antisera to HGH.[a]

	Inhibiting antiserum (unconjugated)	Titer of antiserum[b]	Degree of inhibition[c]		
			Rat	Human	Bovine
1	Hgh-3	1/6400	+	++++	0
2	Hgh-5	1/6400–1/12,800	+	++++	0
3	Hgh-10	1/25,000	++	++++	0
4	Hg-1	1/12,800–1/25,000	++++	++++	++++
5	Hgh-2X	1/6400	0	0	0
6	Hgh-3X	1/6400	0	0	0
7	Hgh-4X	1/25,000	0	++	0
8	Hgh-5X	1/6400–1/12,000	+	++	0
9	Hgh-6X	1/25,000	++	++	0
	Hgh-GP	1/25,000	+	+	0

[a] Fluorescein-conjugated anti-Hg-1 was used for staining.

[b] Titers were determined by BDB-hemagglutination.

[c] ++++ = complete inhibition.

TABLE III. Direct Staining in Pituitary Glands of Various Species with Fluorescein-Conjugated Antisera to HGH.

	Conjugated antisera	Titer of antiserum	Staining (direct method)		
			Human	Rat	Bovine
1	Hg-1	1/25,000	Present	Present	Present
2	Hgh-5	1/6400	Present	Present	Present
6	Hgh-3X	1/6400	Absent	Absent	Absent
7	Hgh-4X	1/25,000	Present	Present	Present
8	Hgh-5X	1/6400	Present	Absent	Absent
10	Hgh-4	1/6400	Present	Present	Present

Inhibition characteristics of an antiserum appears to be independent of titer since, of four antisera of similar titer, three produced staining in the human pituitary and only two in bovine and rat pituitaries by direct method (Table III). By indirect method (Table IV), all antisera produced fluorescence of variable degree in rat and human pituitaries. Antiserum Hgh-3 showed no change in titer after conjugation but produced no staining.

The degree of staining or of inhibition did not correlate with the number of boosters given to the animal during immunization (Table I). It is apparent that each rabbit had chosen a different antigenic group in the same antigen, and that the major antigenic group was to HGH, since almost all antisera produced fluorescence in human pituitary. That some antisera produced specific staining in rat and bovine pituitaries could mean that the minor antigenic groups chosen by the rabbits differed. When the staining and inhibiting antisera were the same, inhibition was complete, suggesting the inhibiting antiserum blocked the same antigenic groups against which the antibody of the staining antiserum was directed. However, when the staining and inhibiting antisera were from different animals, staining was not invariably inhibited, indicating that the inhibiting and staining antisera occupied different sites. In human pituitaries, almost all antisera produced staining as well as inhibition, indicating that they had some common groups that were present in HGH; these probably represented major antigenic determinants in HGH.

TABLE IV. Fluorescent Staining in Human and Rat Pituitary Glands with Various Antisera to HGH.[a]

	Antisera	Degree of staining	
		Human	Rat
1	Hgh-3	++++	++++
3	Hgh-10	++++	++++
5	Hgh-2X	++	++
6	Hgh-3X	++	++
7	Hgh-4X	++++	++++
8	Hgh-5X	++++	++
9	Hgh-6X	++++	++
10	Hgh-4	++++	++++

[a] Staining was by the indirect method, using fluorescein-conjugated sheep anti-rabbit gamma globulin.

The titer obtained by the hemagglutination method is not to a single antigenic group, and the many groups it represents can not be differentiated. Although all of the rabbits used for immunization might choose a common major antigenic determinant, which would be present in all of the antisera, each could choose a different minor antigenic group on HGH and produce antibodies to it. This heterogeneity of antibodies is not peculiar to HGH having previously been demonstrated by Arquilla and his colleagues (3–5) in studies with antisera to insulin.

The experiments with several antisera to HGH indicate that this hormone has multiple antigenic sites and that individual animals choose different antigenic determinants against which to produce antibodies.

1. Nayak, R., Ph.D. thesis, McGill University (1966).

2. Nayak, R., McGarry, E. E. and Beck, J. C., Endocrinology (Accepted for publication).

3. Arquilla, E. R., Alexander, J. D., Placek, E. D., and Loosli, E. S., Diabetes 11, 412 (1962).

4. Arquilla, E. R. and Finn, J., Science 142, 400 (1963).

5. Arquilla, E. R. and Finn, J., J. Exptl. Med. 118, 55 (1963).

6. Raben, M. S., Science 125, 883 (1957).

Received May 27, 1968. P.S.E.B.M., 1968, Vol. 129.

Influence of Fasting on the Formation of Cholesterol Arachidonate by the Serum Cholesterol Esterifying Enzyme (33321)

LEON SWELL AND M. D. LAW

Veterans Administration Hospital and Department of Biochemistry, Medical College of Virginia, Richmond, Virginia 23219

Sperry (1) first demonstrated that human serum contains an enzyme that catalyzes the esterification of free cholesterol. The enzyme is present in other species (rat, dog, and chicken) and has been characterized as an acyl-transferase (2, 3). The cholesterol ester fatty acids are derived from the 2-position of lecithin and are principally polyunsaturated (2). Also the rat plasma esterifying enzyme appears to react preferentially with the high-density lipoproteins that are rich in polyunsaturated esters (4, 5). Portman and Sugano have shown that the types of cholesterol esters synthesized by the plasma-esterifying enzyme is dependent on the source of the plasma enzyme. Fresh rat plasma synthesizes esters rich in arachidonate, while human plasma synthesizes esters rich in linoleate. In the course of studies (7) on the synthesis of the individual serum and cholesterol esters, it was recently shown that the types of cholesterol esters synthesized by the different liver enzyme systems is dependent on the nutritional status of the animal. The liver particulate and soluble cholesterol ester synthetase systems from fasting rats synthesized considerably more arachidonate than those systems from the liver of fed rats. At the same time the serum cholesterol esters of fasting rats contained a much higher proportion of arachidonate and a lower proportion of linoleate than did those of fed animals. In view of these findings, it became of interest to ascertain what effect the nutritional state of the animal would have on the serum-esterifying system. The data presented show that the serum enzyme of the fasting animal synthesizes significantly more arachidonate than that of the fed animal. Also, there are marked changes in the fatty acid composition of the phosphatidyl choline and cholesterol ester fractions.

Methods and Materials. Male rats (Wistar strain), weighing 175–200 g, and fed on a stock pellet chow diet were used. Fed and fasting rats refers to those animals who either had access to food at all times or were fasted 18 hr prior to sacrifice. Blood was obtained from the abdominal aorta and immediately chilled at 2°, allowed to clot, and centrifuged at 2° to separate the serum. Two-milliliter aliquots of serum were incubated with 0.5 μCi (specific activity 131 μCi/mg) of cholesterol-4-^{14}C (added in 0.1-ml acetone) for 1 hr at 37° in a Dubnoff Incu-Shaker. Control digests with serum heated at 60° for 10 min or 20 μmoles *N*-ethyl maleimide gave no enzymatic activity. The incubated and unincubated serum fractions were extracted with 20 vol of 2:1 chloroform methanol (8). The free and esterified cholesterol fractions and phospholipids were separated by silicic acid column chromatography (9). The ^{14}C activity of the cholesterol fractions was determined by liquid scintillation counting. The free and esterified cholesterol concentration was determined by the method of Sperry and Webb (10). The ^{14}C activity distribution among the various cholesterol esters was determined by separating the cholesterol ester fraction into four major cholesterol ester classes (saturated, monounsaturated, linoleate,